Chapter 18

Preserving and Specimen Handling: Insects and other Invertebrates

by

Lars Krogmann
Staatliches Museum für Naturkunde Stuttgart (SMNS)
Rosenstein 1, 70191 Stuttgart, Germany
Email: krogmann.smns@naturkundemuseum-bw.de

Joachim Holstein
Staatliches Museum für Naturkunde Stuttgart (SMNS)
Rosenstein 1, 70191 Stuttgart, Germany
Email: holstein.smns@naturkundemuseum-bw.de

Abstract

Up-to-date field techniques for preserving and handling invertebrate specimens with special emphasis on insects are summarized. Different preservation techniques for specimens sampled for molecular and morphological analyses as well as for natural history collections are presented and hints for scientific labelling of specimens in the field are given. Fluid fixation of molluscs, annelids, nematodes, and plathelmints are briefly discussed. Fluid or dry preservation of insects and other arthropods depends on the purpose of the field work and on the taxonomic groups. The most commonly used killing agents, fixation fluids and sample containers are discussed. Direct pinning and card mounting of insects in the field is explained and hints for transport of specimens are given. The recipes of frequently used reagents and solutions are listed in an appendix.

Keywords: specimen sampling, killing, labelling, transport, fluid preservation, dry preservation, direct pinning, card mounting

1. Introduction

This chapter will discuss the steps following immediately after the collecting event. The first consideration is what is the purpose of the fieldwork, *e.g.,* what is supposed to be done with the collected invertebrate specimens. If the specimens shall not be kept alive for experimental studies or for rearing larvae to adults (this will not be dealt with in this chapter) the first logical step is the killing or in some cases the narcotisation of the specimens. If traps like Malaise traps, flight interception traps, yellow pan traps or pit fall traps have been used this step can be skipped in most cases as the specimens are normally killed by a fluid with which the collecting containers in the traps were filled. When the specimens have been collected alive it must be considered if the specimens will be used as a whole, *i.e.* as voucher specimens in natural history museums or if they shall wholly or partly be used for molecular or morphological investigation.

2. Specimen sampling and labelling

2.1. Specimen sampling for molecular analyses

Molecular analyses, such as DNA sequencing, require particular considerations that preserve DNA and are only briefly considered here. Usually the best option to preserve the DNA for long time storage is to transfer the specimens to high percentage ethanol (95-99%) which serves as both, as killing and fixation agent. A second option is to keep the specimens alive during transport and have them fresh frozen in the laboratory. This allows storing suitable DNA for decades. The biggest enemy of DNA is humidity so long time storage in low percentage ethanol (70%) should be avoided as well as leaving the specimens in moist atmosphere. Specimens preserved in ethanol should be put in dark and cool conditions as soon as possible and not left out in daylight during fieldwork (or in the laboratory!). This applies to all invertebrate specimens sampled in ethanol whether they are to be used for DNA studies or not. If only low percentage ethanol is available it may a better option to kill the specimens with a killing agent, let them dry quickly and store them (or just selected body parts of them) in the freezer and/or high percentage ethanol in the laboratory. Even if the specimens are stored in dry collections they usually allow extracting suitable DNA for ten or more years (in some cases even hundreds of years) but it seems that the success rate decreases significantly over time.

2.2. Specimen sampling for morphological analyses

If specimens shall be used for morphological investigation (*e.g.*, anatomical dissections, thin-sectioning) it is generally most appropriate to kill and store them directly in a fixation fluid. The fluid normally depends on the taxonomic group and/or the morphological analyses. Specimens that will be used for histological work can be preserved in a number of different fluids that often contain formalin. Fluids like Kahle's or Bouin's solution (Appendix 1) are the best choice for insect larvae as they fix tissues. Kahle's solution also prevents larvae from discoloration while Bouin's solution may change the colour of larvae to light yellow. Before formalin is used it should be considered that it contains

formaldehyde which cross-links proteins and makes tissue samples unusable for DNA extraction.

2.3. Specimen sampling for natural history collections

In the majority of cases specimens are collected to be stored in natural history collections for documentation and research. Even though this does not preclude that parts of the specimens may still be used in future molecular or morphological studies, the primary purpose of the fieldwork is to yield specimens that should be preserved and stored as a whole. The question that arises is if the specimens are to be stored in a dry or a wet collection (*i.e.* usually an ethanol collection). In most cases specimens that are to be stored in an ethanol collection will already be killed and fixated in ethanol directly in the field (70-80% is the standard ethanol concentration). It can be suitable to add a small amount of glycerol to the ethanol, which makes the specimens less stiff. Also glycerol does not evaporate which can be an advantage when containers do not close hermetically. However, use of glycerol should be avoided for small winged insects, such as Micro-Hymenoptera as it complicates the subsequent dry-mounting of these specimens. Glycerol softens the wings in small insect specimens excessively, so that they will not stay flat when specimens are air or critical point dried and card-mounted. Only for small winged insects that need to be slide-mounted (*e.g.* Thysanoptera), glycerol-ethanol solutions are a good option. Ethanol vials should be completely filled which makes specimens less prone to damage during transport. Even small air bubbles that slosh around in the vials can cause damage to very fragile specimens, so special care should be taken to minimize these risks in the field. It should be considered that glass vials that are completely filled with ethanol may crack or even explode in the hold of an aeroplane. With plastic vials these problems can be overcome but it is still useful to seal the screw-cap of the vial with stripes of Parafilm® as it may become loose or undone during transit. An authorization is needed to transport ethanol in an aeroplane and therefore dry storage of specimens (see 3.3.4) during transit is more advisable. Specimens that are to be deposited in a dry collection are normally killed by a gaseous killing agent and stored dry before they are further processed (*e.g.,* pinned and mounted). Keeping and storing specimens dry in the field usually requires more care from the collector as specimens are more fragile and prone to damage compared to specimens preserved and transported in a fluid fixation agent. This is even more severe when specimens are completely dried, which can occur within a few hours on a hot and dry collecting day. Especially dry insects are very delicate and care must be taken to prevent specimens from loosing legs, heads or antennae during transport. Many collectors therefore transport the specimens in a moist atmosphere, which can be a plastic box that is laid out with wet tissue. A few drops of thymol-camphor solution (Appendix 1) or a few crumbs of crystalline thymol should be added to the tissue to prevent the specimens from moulding. If smaller specimens numbers are collected it may also be appropriate to pin the specimens directly after collecting (*e.g.,* in the field or immediately after in the hotel or field station) which secures specimens and facilitates subsequent preparations. Special transport boxes can be obtained from entomological suppliers. Also in these dry boxes it is appropriate to add thymol as larger specimens that cannot dry fast may get mouldy.

2.4. Labelling

Even experienced biologists tend to inappropriately label specimens so this step needs special attention as biological specimens loose their significance for research and documentation if they are not or insufficiently labelled. Labelling should be done in the field, directly after collecting the specimens or after emptying the traps. It can be convenient to prepare the labels in advance and already print parts of the information (*e.g.*, parts of the locality data, name of collector) beforehand and just add the specific data (*e.g.*, date and altitude) in the field. External labelling of tubes or transport boxes can be useful but does not replace a proper labelling of the individual specimens or samples inside the respective container. The most widespread mistake during fieldwork is to just add numbers to the specimens and to list the collection data on separate sheets. Even though the collector has strong intentions to properly label his samples "some when" after the fieldwork there is always a high risk that this will never happen and that the collected specimens will loose their scientific value.

It is crucial that specimens are labelled with all necessary collection data:

- **Locality** (Country, Province, nearest City, Region)
- **Name of project** (if available)
- **GPS data** (if available)
- **Altitude**
- **Collecting method**
- **Date of collection**
- **Name of collector**

Further data (*e.g.,* habitat type, host plant, weather, and temperature) should also be added on (an) additional label(s). The golden rules (Table 1) should be followed to minimize the risks of mixing samples or loosing locality information.

Labels for dry specimens should be written with water proof pens or pencils. Labels for ethanol vials should preferably be written with ethanol-proof ink, *e.g.*, Micron archival ink pens (SAKURA corp.) or alternatively with a pencil. Laser printed labels will not last in ethanol and should not be used. Wet preserved samples should be generally labelled on tight paper which is not negatively affected by the fixation agent. Handwritten labels can later be replaced by proper type-written labels in the laboratory but care needs to be taken that spelling mistakes are avoided. Long-term storage and viability of ink on collection labels is a big challenge for curators of natural history collections and cannot be addressed in this field manual. However, every collector should make sure (prior to collecting) that long term storage of his natural history specimens and the necessary curatorial care can be guaranteed by the respective institute.

Rule 1	Always label the specimens and add the collection information to the specimens immediately (*i.e.*, directly in the field).
Rule 2	Preferably every specimen gets an individual label, but if this cannot be achieved due to high specimen numbers at least every sample gets an individual label.
Rule 3	A sample contains only specimens which have identical collecting data and which are clearly separated in an individual container from the other samples.
Rule 4	If specimens are pre-sorted into smaller samples every sample needs to get a proper label.
Rule 5	Labels are always placed inside the vials, labelling the vials just from the outside is insufficient.
Rule 6	Numbering of samples does not replace locality labels and may later result in confusion and loss of information.

Table 1. The six golden rules for labelling scientific specimens in the field.

3. Invertebrate taxa

The right treatment of collected invertebrate specimens is not only dependent on the purpose of the collecting (see 2.1-2.3) but also on the invertebrate taxon and its life history stage. Soft bodied invertebrates generally require fixation as they suffer from shrinkage if air-dried while hard bodied, sclerotized invertebrates can often be air-dried and may even be damaged if put in ethanol. However, there are many exemptions from this rule and many taxa require a special treatment which made it necessary to devote a separate chapter to the different terrestrial and limnic invertebrates that can be the subject of fieldwork.

3.1. Molluscs (Mollusca)

If the soft parts of the animals shall be preserved as well as the shell (if present), it is necessary to narcotize the specimens prior to the killing. This ensures that the organisms are expanded and fully display their characteristic features. For this, terrestrial gastropods are best placed in a jar of water. The animals will die in a relaxed position (outside the shell if it is present) within 1 or 2 days (Sturm *et al.*, 2006). Afterwards the specimens should be transferred into a preservative, which can be 80% ethanol, a mixture of ethanol (80%), water (15%) and glycerol (5%), or formalin. Several different preservation methods have been described for molluscs (see Piechocki & Händel, 1996; Sturm *et al.*, 2006) but not all of them are practicable for field trips.

Molluscs, which are anticipated to be included in DNA studies, should be transferred immediately after collecting into 95-99% ethanol.

3.2. Round worms (Nematoda), flat worms (Plathelminthes), and segmented worms (Annelida)

Nematodes are usually killed and preserved in the laboratory after they have been extracted from plant or animal tissue or from soil samples. Due to their small body size, nematodes are always handled in fluid medium under a dissection microscope. General techniques for handling, killing and preserving nematodes are summarized in Kleynhans (1999).

Flat worms are a diverse group of organisms from which only the Turbellaria contain non-parasitic groups. The parasitic groups are not included here but their preservation is discussed in Piechocki & Händel (1996). Aquatic and terrestrial Turbellaria are best preserved in FAA (Appendix 1). Alternatively, formalin (5%) can be used as a suitable fixation agent. The problems of specimen contracting can be overcome by a variety of techniques which are discussed in Knudsen (1972) and Piechocki & Händel (1996). Some of these techniques require the use of mercuric chlorides which we would not recommend (especially not in the field) due to its toxic nature. Final storage of the flatworms should be in formalin (5%) or in ethanol (70-80%).

From the segmented worms (Annelida), only free living earthworms (Oligochaeta: Lumbricidae) and leeches (Hirudinea) are dealt with here. Oligochaete worms shall not be placed immediately in ethanol (unless they are to be used for molecular study) as they shrink. The specimens are washed in a shallow dish and killed in a weak formalin solution (1-2%). It is important to slew the specimens with forceps constantly in the formalin solution which limits the number of specimens that can be dealt with to about five specimens per treatment. After the oligochaete worms got immobilized they are stretched outside the solution. The specimens are then placed on blotting paper and permanently wetted with formalin solution. Alternatively, specimens can be covered by cellulose which has been imbued with formalin. After the specimens hardened (after 30-40 minutes) they need to be transferred into glass vials, which should be long enough to house the specimens. The vials can be either filled with formalin solution (5%) or ethanol (70-80%). Leaches are narcotized in 5-15% ethanol until they do not show any reactions anymore. This may take ½ to six hours depending on the size and physiological condition of the specimen. Fixation occurs in formalin solution (1:4), ethanol (70%) or formol-alcohol (Appendix 1).

3.3. Arthropods (Arthropoda)

Arthropods are the most diverse group of terrestrial organisms and their overall abundance and diversity makes them an important target group for fieldwork. Soft bodied arthropods are best transferred by spring steel forceps which allows safe handling without damaging the specimens (Fig. 1). Small, hard bodied arthropods are best handled or divided into smaller samples with the help of an aspirator (Fig. 1). In the following we will discuss different preservation methods for arthropods, which are best practicable in the field.

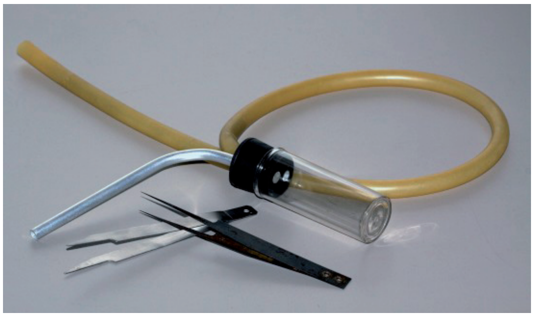


Fig. 1. Handling arthropods in the field. Delicate, soft bodied specimens can be handled with spring steel forceps. Minute, hard bodied specimens are best transferred by an aspirator which can be obtained from entomological suppliers. (Photo by authors).

3.3.1. Fluid preservation of arthropods

There can be a difference between the collecting and fixation fluids which are used for fieldwork and fluids which are used for permanent preservation in natural history collections. Here we only discuss those methods which are used during fieldwork, *i.e*. which concern the collecting and short-term storage during transport. In most cases specimens are directly killed and preserved in the same fixation agent but sometimes the killing agent can differ from the fixation agent. For example, it can be more suitable to collect (and kill) arthropods in water or salt water (*e.g.*, in a pan trap) as ethanol or other fixation agents may act as repellent or attractant thus artificially altering the species composition and diversity of the samples. However the time specimens are kept in non-fixation agents must be held to a minimum as specimens will start decaying within 1-2 days (depending on the temperature).

Standard fixation fluids for arthropods are:

- 70 -80% ethanol (higher alcohol concentration should only be used when specimens are to be included in molecular investigations). This is by far the most common fixation agent and suitable for the vast majority of arthropods.

- 2% acetic acid (like the concentration of vinegar), also feasible for permanent preservation. It is normally used for well sclerotized taxa like Coleoptera, Heteroptera or ants, which shall be dry- mounted in the laboratory. The specimens stay soft and elastic and normally do not need to be relaxed prior to dry-mounting. However, acetic acid is not feasible for most Arachnida and Crustacea because the specimens become too soft and Crustacea will even loose their integumental calcium deposits.

- Acetic acid-glycerol-alcohol solution (AGA) is suitable for small wingless arthropods such as mites (Acari) and for small winged forms like thrips (Thysanoptera). AGA is not suitable for winged forms that are intended to be dry-mounted.

- Lactic alcohol is suitable for aphids (Aphidoidea) and scale insects (Coccoidea).

- Saturated picric acid solution (odourless, only used as fixation liquid, sample has to be transferred into ethanol afterwards). A negative side effect is that specimens fade into yellow according to the luminous yellow colour of the picric acid.

The standard preservation fluid for short and long term storage of arthropods is 70 -80% ethanol. There are a number of different vials available from which those with a screw top should be preferred. Glass vials (Fig. 2) are commonly used but during field work they always bear the risk of being broken, which may lead the specimens to be lost and the collector to be injured by scattering glass pieces. The best option for field work is to use transparent plastic vials with screw tops (Fig. 3). Alternative preservation fluids which may depend on the taxonomic group or the purpose of the field work can be found in Appendix 1. In general, formalin is not recommended for collecting and preserving arthropods. Specimens become very rigid which complicates the handling. In some cases

this effect may be desirable, as for soft-shelled specimens or larval instars or for specimens that are intended to be included in anatomical dissections.



Fig. 2. Handling Glass vials are not a good choice for storing specimens during field work as they are heavier and less safe than plastic vials. (Photo by authors).



Fig. 3. Plastic vials are safer than glass vials during field work and also less heavy. A screw top with a ring gasket tightly closes the vials and prevents evaporation of the ethanol. The transparency of the vials allows the collector to check the content and labels without the need to re-open the vials. (Photo by authors).

3.3.2. Dry-mounting of insects after fluid fixation

The used fluid can be of great importance if the collector's intention is to dry-mount specimens after fixation in a preservation fluid. Acetic acid (2%) is recommended for well sclerotised taxa like beetles (Coleoptera), bugs (Heteroptera), and ants (Formicidae). Specimens in ethanol mostly become rigid and handling and mounting is complicated. Better results are only accessible via more elaborate methods like chemical treatment, heat impact or critical point drying. Inapplicable for dry-mounting after fluid fixation are Lepidoptera, Diptera, as well as pilose and coated Hymenoptera. Micro-Hymenoptera as well as any other small and delicate insect specimens should only be mounted after critical point drying. In these cases it is necessary to transfer them along an ethanol series in the laboratory, in which the ethanol concentration is gradually increased from 70-80% via 90% and 95% to absolute ethanol. Also for some insect larvae and small arachnids it can be more advisable to mount them after critical point drying instead of storing them permanently in ethanol.

3.3.3. Standard methods for dry preservation and mounting of insects

The standard method for dry preservation of well sclerotised insects is the use of specific insect pins. All characters of the specimen should be readily visible by mounting it in a characteristic manner like spreading wings and limbs. There are some different setting and mounting recommendations according to the taxonomic group to be mounted.

Dry-mounting and pinning is recommended or even necessary for the following groups:

- Lepidoptera
- Coleoptera
- Hymenoptera
- Diptera (partim: most Brachycera, single Nematocera groups)
- Heteroptera
- Saltatoria and other “Orthoptera“
- Odonata (imagines and exuviae)
- Neuropterida (partim)

All other taxonomic insect groups as well as insect larvae, Arachnida, Myriapoda, and Crustacea are best killed and preserved in 70 -80% ethanol.

There are 3 established alternatives of pinning (with some modifications in special cases) which are determined by the specimens' dimensions:

- Direct pinning of the specimen with commercially available insect pins of different size for well dimensioned objects.

- Direct pinning of the specimen with commercially available minuten pins of different sizes for small specimens (standard method for Micro-Lepidoptera). Minuten pins are to be pinned into small double mount strips, which are again pinned with common insect pins (see Schauff, undated, for more information).
- Gluing the specimens onto small paper cards of different size and shape. This method is also referred to as card-mounting.

Direct pinning of specimens should be done with an insect pin that fits to the specimen's size. The standard size for insect pins is 1 or 2 which fits for most Lepidoptera, large Hymenoptera and many Coleoptera. Larger specimens should be pinned with size 3, 4 or 5, while for smaller specimens, pins with size 0, 00, or even 000 are available. However, it should be noted that pins with size 0 or smaller are difficult to handle. Pinning through the labels or through the paper layer of insect boxes should be done with great care as the thin pins are easily twisted. Therefore, it may be more appropriate to use minute pins or glue for very small specimens. Direct pinning of insects should be done in a way that the pin is in a right angle to the body (Fig. 4). The insect specimens should rest about 1/3 of the pin length away from the top. This gives enough space to handle the specimens, *i.e.* to grip the top of the needle by the thumb and the index finger without damaging the specimens with the fingertips or fingernails. The specimens should not rest further away from the top of the needle as the bottom space is needed for collection and determination labels. The pin is usually inserted through the mesothorax but the exact insertion point depends on the insect group (Figs 5A-D). Bugs (Heteroptera) are pinned submedially through the scutellum (Fig. 5A). In Hymenoptera and Diptera the insertion point is slightly removed laterally from the median axis (Fig. 5B). This allows median sculpture or bristle patterns to remain intact and visible medially and also on one side. Beetles (Coleoptera) are pinned through the right elytron (Fig. 5C). In butterflies and moths (Lepidoptera) the insertion point is in the middle of the mesothorax (Fig. 5D).

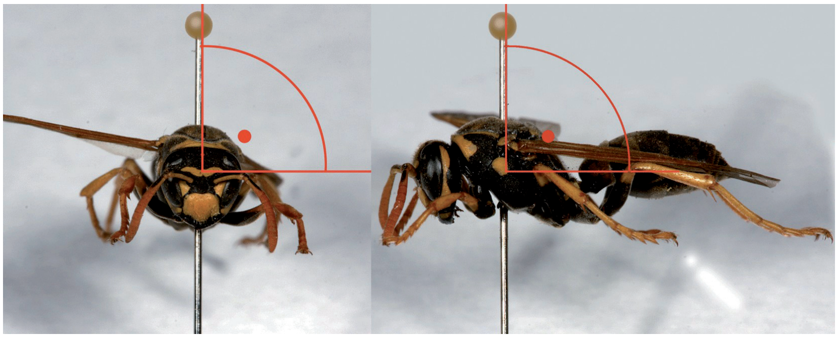


Fig. 4. While pinning an insect specimen care must be taken that the needle is in a right angle to the body of the insect. This needs to be checked in frontal and lateral view. (Photo by authors).

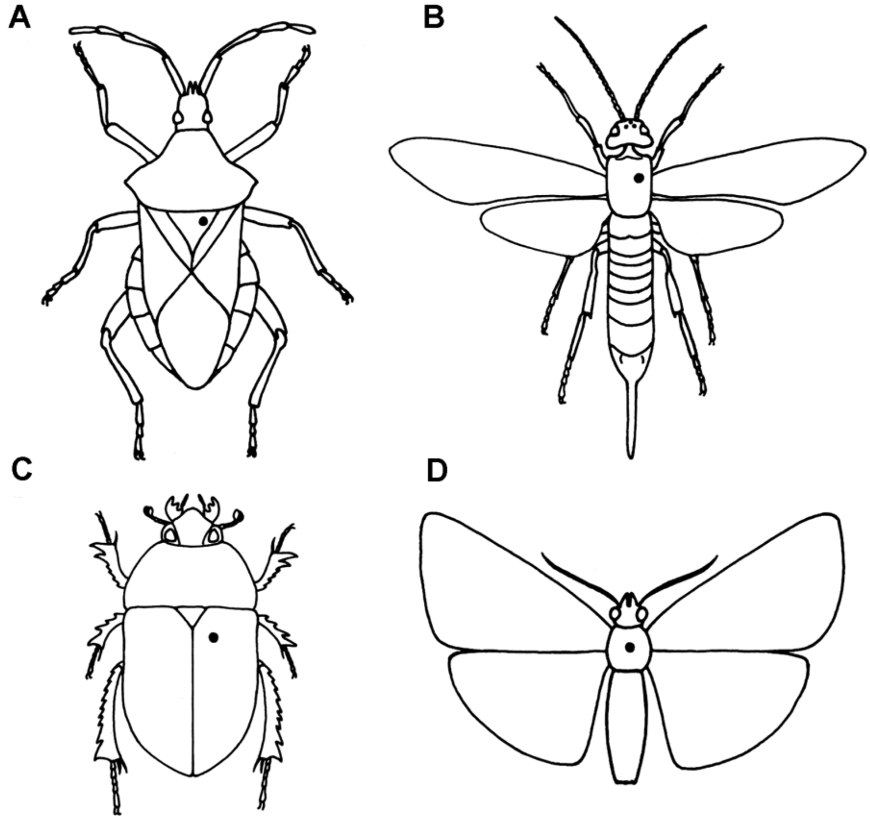


Fig. 5. Insertion of the insect pin, as exemplified in the orders of A. Heteroptera; B. Hymenoptera; C. Coleoptera; and D. Lepidoptera (after Abraham, 1991). See text for more details.

Very small insects (body length below 3 mm) should never be directly pinned on minutens as specimens will always be damaged or lost over time. Card-mounting is the method of choice for small beetles, bugs and Micro-Hymenoptera. Beetles and bugs are usually glued on rectangular cards (Fig. 6). Small Hymenoptera can either be glued on rectangular cards or on the tip of card points, which are small triangles of stiff paper (Fig. 7) (Noyes, 1982, 2009). The latter method has the advantage that the specimen can also be observed in ventral view. The paper cards with the mounted insects are pinned with common insect pins of larger size (sizes 3 to 5). Pins of that size can easily be inserted through the paper cards. The glue should be water-soluble or ethanol soluble so that specimens can easily be removed from the card in case they need to be re-mounted without being damaged. Noyes (2009) recommends glues, which were made from animal products. The best option is to use shellac, a resin produced by lac bugs (Coccoidea). It is important to use shellac (or any other glue) in the right solution, *i.e*. the glue should not be too thin (the specimen will sink in the glue) or too thick (the specimen will not attach tightly enough). Shellac can easily be brought to the right viscosity by adding drops of ethanol or by letting part of the ethanol evaporate from the glass tube in which

the resin is deposited. Shellac is commonly used in North America but less widespread among European entomologists. Shellac resin can be obtained from entomological suppliers in the United States. However, even if we would recommend shellac over other glues, there are also a few drawbacks of shellac which are best summarized in Noyes (2009). Seccotine (fish glue) is a water-soluble glue and a good alternative to shellac. Noyes (pers. comm.) recommends the use of shellac for card points and seccotine for card rectangles. For long and slender insect groups, *e.g.* ichneumonid wasps, it can be an alternative to glue them on to the side of an insect pin with shellac.



Fig. 6. Card mounting is the method of choice for small sized insects. Small beetles are usually mounted and glued on rectangular paper cards, which are available in various sizes from entomological suppliers. (Photo by authors).



Fig. 7. Card points are small triangles of stiff paper that allow specimens to be observed from all sites if specimens are glued laterally to the tip of the triangle. This is a suitable method for mounting Micro-Hymenoptera. (Photo by authors).

Accepted killing methods for arthropods to be dry-mounted are:

- Jar, containing absorbent paper saturated with ethyl acetate (Fig. 8B). This method is suitable for most insects apart from Lepidoptera. Avoid too wet content of the jar because of possible agglutination of small, pilose, or coated specimens.
- Potassium cyanide inside a killing jar (Fig. 8D). This is best method for Lepidoptera, except for some resistant groups like Zygaenidae moths. It is also feasible for most other taxonomic groups. Safety regulations are essential to avoid intoxication! It is the responsibility of the collector to make sure that the killing jars are always kept under supervision and do not get into the hands of others!
- Ammonium chloride, injected via syringe (Fig. 8C). This can be used for larger butterflies and moths effecting rapid killing and for softening rigour mortis.
- Freezing.

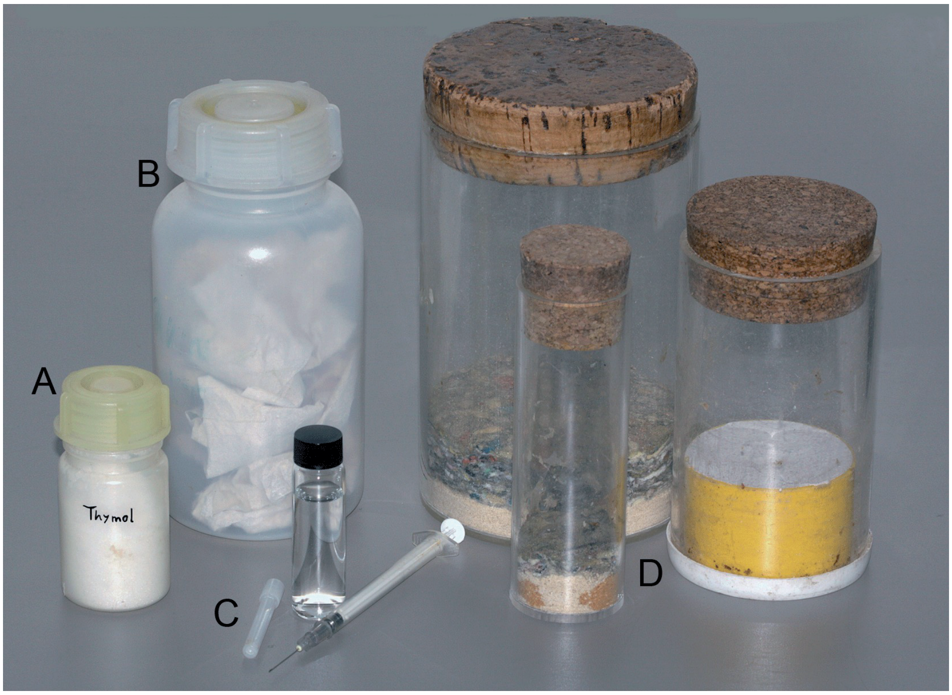


Fig. 8. Devices against moulding and for killing specimens. A. Thymol prevents specimens from fungal damage and can be applied crystalline or in a solution; B. Ethyl acetate is used for most insects apart from Lepidoptera; C. Ammonium chloride is used for larger Lepidoptera and injected by a syringe; D. Killing jars containing potassium cyanide can be purchased from entomological suppliers in various sizes. Specimens are usually killed in smaller jars and transferred into a large jar after they are narcotized. Layers of tissue between the specimens prevent them from mechanical damage during fieldwork. (Photo by authors).

3.3.4. Preservation in the field and transport

If time availability and the amount of samples permit, it is good practice to mount or prepare for dry preservation.

Even if this is not possible, the right preparations and transportation conditions are essential for best quality of set specimens. When pinning and mounting sheetings and/or setting boards, transport and store boxes are available they should be used already in the field. Protected and dry storage is important; especially intruding ants could be fatal. In many cases setting in the field is not possible. In these cases, the following recommendations should be noted to facilitate later setting and mounting:

- Use cardboard tubes of different diameter for transport (Fig. 9). Both sides of a tube are to be closed with a cotton plug. The freshly killed sample is placed directly inside the tube. It can be transferred into a soften chamber afterwards for preparation of setting and mounting. Check adequate labelling! This method is feasible for strongly sclerotized specimens (*e.g.*, beetles) which need to be stored during fieldwork before they can be dry-mounted in the laboratory. However, scaled, pilose, and coated specimens could be rubbed off during transport. Cardboard tubes are preferred over glass or plastic ones as they are lightweight, fracture-proof and absorb moisture. The tubes are to be stored inside of feasible sealed transport boxes containing crumbs of thymol (Fig. 8A) against moulding.

- Use butterfly envelopes of different size, made of vellum (Figs 10, 11). This is the best method to transport or even store dry unset Macro-Lepidoptera, but also feasible for other winged insect orders like Odonata and Neuroptera. It is important to “close” the specimens inside of the envelope with the wings folded upwards. This protects the more important upper sides of the wings (as identification characters) against rubbing and facilitates later setting and spreading. If this is not possible in case of *rigor mortis*, the specimens have to be injected by syringe with ammonium chloride to soften *rigor*. Placing more than one specimen into one envelope should be avoided as they may damage each other during transport. Every single envelope has to be labelled individually with the full locality data! The envelopes are to be stored inside of sealed transport boxes containing crumbs of thymol against moulding (Fig. 11). For softening the specimens the whole envelope has to be transferred into the soften chamber without removing its content.

- Use small plastic boxes laid out with layers of cellulose wadding. Freshly killed insect specimens can be placed between the layers and will be protected during transport. Thymol should be added against fungal damage.

- Use transport boxes with plastazote foam pinning bottoms (Fig. 12). Insects can be pinned without setting and plunged into the box in a space-saving manner. For later setting, mounting or spreading, they can be softened easily inside a soften chamber. This is feasible for all well sized specimens, which are to be pinned and set. Adding labels to every single specimen is essential! Fragile Micro-Lepidoptera that cannot be transported on setting boards (this is the preferred option) should be pinned directly onto plastazote in small transport boxes (Upton, 1991). Spreading the wings with

minuten pins can easily be done in the field and the roughness of the plastazote will hold the wings in place (Fig. 12). This method does not replace proper spreading on a setting board but will greatly facilitate this as the wings are already partially spread.



Fig. 9. Cardboard tubes are ideal for hard bodied insects, such as beetles. Specimens are ideally protected during transport and less prone to moulding as the tubes absorb moisture. (Photo by authors).

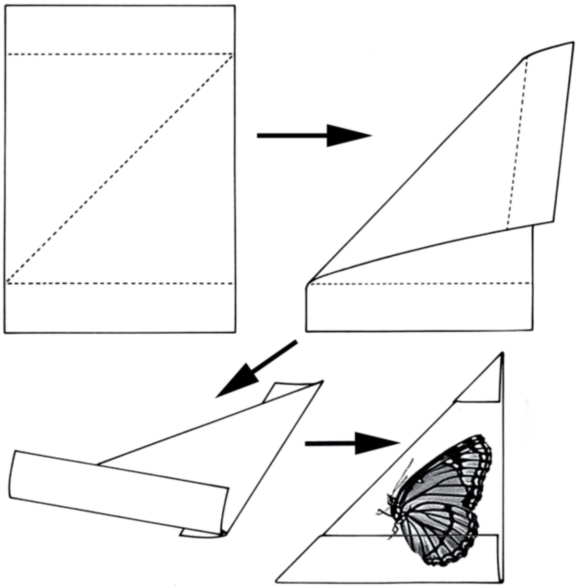


Fig. 10. Envelopes for storing insect groups, such as Lepidoptera can be easily folded from rectangular paper (after Abraham, 1991).



Fig. 11. Vellum envelopes are a simple option for storing Lepidoptera specimens as they do not need to be hand-folded but can be readily purchased in various sizes from philately purchasers. The envelopes are best stored in tightly lidded boxes which can be laid out with wet cotton. The moist atmosphere keeps the specimens relaxed before mounting. Thymol must be added to prevent moulding. (Photo by authors).



Fig. 12. Transport boxes with plastazote foam pinning bottoms are ideally suited to transport pinned insects in the field. Fragile Micro-Lepidoptera that cannot be transported on setting boards should be pinned directly with minutens onto the plastazote. The wings should be spread and the roughness of the plastazote will hold them in place. This greatly facilitates later spreading on a setting board. (Photo by authors).

4. Acknowledgements

We thank Daniel Bartsch and Axel Steiner (Staatliches Museum für Naturkunde Stuttgart) for helpful discussions and advise on preservation of Lepidoptera.

5. References

ABRAHAM, R. 1991. *Fang und Präparation wirbelloser Tiere*. G. Fischer, Stuttgart, New York: 132 pp.

KLEYNHANS, K.P.N. 1999. *Collecting and preserving nematodes. A manual for nematology*. ARC Plant Protection Research Institute, Pretoria, South Africa: 54 pp. Available at: http://spc.int/pps/SAFRINET/nem-scr.pdf; accessed 28 September 2009.

KNUDSEN, J.W. 1972. *Collecting and preserving plants and animals*. Harper and Row, New York, Evanston, San Fransisco, London: 320 pp.

NOYES, J.S. 1982. Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). *Journal of Natural History* 16: 315-334.

NOYES, J.S. 2009. Universal Chalcidoidea Database. Collecting and preserving chalcidoids.http://www.nhm.ac.uk/researchcuration/research/projects/chalcidoids/collecting1.html; accessed 28 September 2009.

PIECHOCKI, R. & HÄNDEL, J. 2007. *Makroskopische Präparationstechnik Wirbellose. Leitfaden für das Sammeln, Präparieren und Konservieren*. 5th Ed. E. Schweizerbart, Stuttgart: 346 pp.

SCHAUFF, M.E. undated. Collecting and preserving of insects and mites. Techniques and Tools. http://www.ars.usda.gov/SP2UserFiles/ad_hoc/12754100CollectingandPreservingInsectsandMites/collpres.pdf; accessed 28 September 2009.

STURM, C.F., MAYHEW, R. & BALES, B.R. 2006. Field and laboratory methods in malacology. *In*: STURM, C.F., PEARCE, T.A. & VALDES, A. (Eds) *The molluscs: A guide to their study, collection, and preservation*. Universal publishers, Boca Raton, FL, USA: 445 pp.

UPTON, M.S. 1991. *Methods for collecting, preserving, and studying insects and allied forms*. 4th Ed. The Australian Entomological Society, Brisbane: 86 pp.

6. Appendix I: Reagents and solutions suitable for field work

Acetic acid-glycerol-alcohol solution (AGA) (fixation of small arthropods and thrips)
1 part of glacial acetic acid
6 parts of ethanol (95%)
4 parts of $H_2 0$
1 part of glycerol

Alcoholic thymol-camphor solution (prevention of mould)
100 ml ethanol (96%)
5 g camphor (crystalline)
10 g thymol (crystalline)

Bouin's solution (fixation of insect larvae for histological work)
70 parts of picric acid solution
25 parts of formalin
5 parts of glacial acetic acid

Formal-acetic-alcohol (FAA) (fixation of flatworms and other animals)
10 parts of formaldehyde solution (saturated) (= 100% formalin solution)
50 parts of ethanol (95%)
2 parts of acetic acid
40 parts of $H_2 0$

Formalin
Refers to a saturated solution of formaldehyde. Formaldehyde comes in a saturated solution of 39-40% which equals a 100% formalin solution. That means that *e.g.* a 10% percent formalin solution can be obtained by adding 1 part of formaldehyde (saturated) to 9 parts of water.

Formol-alcohol (fixation of some annelids)
1 part formol
2 parts ethanol (80%)

Kahle's solution (= Pampel's fluid) (general fixation of insect larvae)
30 ml ethanol (95%)
10 ml formalin (35-40%)
2 ml glacial acetic acid
60 ml $H_2 0$

Lactic alcohol (for aphids and scale insects)
2 parts of ethanol (95%)
1 part of lactic acid (75%)